CRYPTOCOCCAL INFECTIONS IN NON-HIV-INFECTED PATIENTS

PETER G. PAPPAS, MD, FACP

BIRMINGHAM, ALABAMA

ABSTRACT

Infections due to *Cryptococcus* species occur globally and in a wide variety of hosts, ranging from those who are severely immunosuppressed to those who have phenotypically "normal" immune systems. Approximately 1 million cases of cryptococcosis occur throughout the world, and is it estimated that there are 650,000 associated deaths annually. Most of these cases occur among patients with advanced HIV disease, but a growing number occur among solid organ transplant recipients and others receiving exogenous immunosuppression, patients with innate and acquired immunodeficiency, and otherwise immunologically normal hosts. Much of our recent knowledge is solely derived from clinical experience over the last 2 to 3 decades of cryptococcosis among HIV-infected patients. However, based on recent observations, it is clear that there are substantial differences in the epidemiology, clinical features, approaches to therapy, and outcome when comparing HIV-infected to non–HIV-infected individuals who have cryptococcosis. If one carefully examines cryptococcosis in the three largest subgroups of patients based on host immune status, specifically, those with HIV, solid organ transplant recipients, and those who are non-HIV, non-transplant (NHNT) infected persons, then one can observe very different risks for infection, varied clinical presentations, long-term complications, mortality, and approaches to therapy. This article focuses on cryptococcosis in the non–HIV-infected patient, including a brief review of ongoing events in the Pacific Northwest of the United States and Canada relative to the outbreak of *Cryptococcus gattii* infections among a largely immunologically normal population, and highlights some of the key insights and questions which have emerged as a result of these important new observations.

INTRODUCTION

Cryptococcosis is an important opportunistic fungal infection causing an estimated 1 million cases and 625,000 deaths per year due to

Correspondence and reprint requests: Peter G. Pappas, MD, FACP, Division of Infectious Diseases, University of Alabama at Birmingham, 1900 University Blvd, 229 THT, Birmingham, AL 35294-0006, Tel: 205-934-9951, Fax: 205-934-5155, E-mail: pappas@uab.edu.

Potential Conflicts of Interest: None disclosed.

central nervous system (CNS) disease among patients with human immunodeficiency virus worldwide (1). The vast majority of cases globally were caused by *Cryptococcus neoformans,* compared to the more geographically restricted *Cryptococcus gattii*. Although cryptococcosis is most often associated with HIV infection, in many centers, especially in more developed countries, the majority of cases occur among non–HIV-infected individuals including transplant recipients; patients who are receiving immunosuppressive agents such as glucocorticosteroids, cytotoxic chemotherapy, TNF-α inhibitors, and other disease modifying agents; and a heterogeneous group of patients with underlying disorders such as organ failure syndromes, innate immunologic problems, common variable immunodeficiency, and hematologic disorders. Moreover, in many centers, up to 20% of cases of cryptococcosis occur in phenotypically "normal" or otherwise clinically non-immunocompromised patients (2).

In the United States, Australia, and Canada, in particular, the decline of HIV-associated cryptococcosis due to potent intervention with combination antiretroviral therapy has led to more focus on non–HIV-associated cryptococcosis including its epidemiology, early recognition, treatment, and outcome (2–5). Moreover, the recent emergence of *Cryptococcus gattii* in British Columbia and the US Pacific Northwest, mostly among otherwise non-immunocompromised individuals, emphasizes the importance in differentiating infection in HIV- and non–HIV-infected patients in hopes of better understanding the clinical features and outcomes among these two groups of patients (6–9). Cryptococcosis in non-immunocompromised patients presents unique diagnostic and therapeutic challenges, and detailed data addressing these differences are lacking. A better understanding of clinical and epidemiological manifestations of cryptococcosis based on host immune status could lead to important new insights into the pathogenesis, immune response, early recognition, treatment, and the prevention of complicated cryptococcal infections.

MICROBIOLOGY AND EPIDEMIOLOGY

Fungi belonging to the genus *Cryptococcus* are basidiomycetes that are encapsulated yeasts. *Cryptococcus neoformans* and *Cryptococcus gattii* are the chief pathogens in humans, and inhalation is the usual route of primary infection (10). *C. neoformans* was originally classified into serotypes A, B, C, D, and AD based on capsular agglutination reactions (11). More recently, *C. neoformans* has been divided into two varieties: *C. neoformans* var. *grubii* (formerly group A) and *C. neofor-*

mans var. *neoformans* (formerly group D) (12). *C. neoformans* is a ubiquitous pathogen found in most temperate regions of the world. It was originally discovered by Sanfelice in 1894 (13), but is commonly found in decaying organic matter and in many soil types, particularly that which has been enriched by animal and bird droppings.

C. gattii (formerly groups B and C) (14) can be divided into four molecular types including VGI, VGII, VGIII, and VGIV (15). Types VGII can be further divided into VGIIa, VGIIb, and VGIIc subtypes (16). *C. gattii* can be readily differentiated from *C. neoformans* by plating the isolate on canavanine-glycine-bromothymol (CGB) agar (17). CGB agar turns blue in the presence of this organism. *C. gattii* is traditionally found in tropical and subtropical geographic regions (18, 19). In 1999, an unprecedented outbreak occurred in British Columbia (BC), Canada (6, 20), and subsequently spread to much of the US Pacific Northwest (PNW) (7–9). Although many hypotheses have been proposed regarding the source of the outbreak and reasons for it dissemination environmentally, to date, the causes remain unclear.

CRYPTOCOCCOSIS IN TRANSPLANT RECIPIENTS

Prospective data derived from the Transplant Associated Infection Surveillance Network (TRANSNET), a consortium of 23 transplantation centers in the United States, showed that cryptococcosis is the third most common invasive fungal infection among solid organ transplant (SOT) recipients. The cumulative incidence over the life of the patient approximates 1% to 2%, but the 12-month incidence in this study was only 0.2% (21). CNS involvement and disease limited to the lungs were observed in 45% and 39% of cases, respectively; the observed 12-month survival in this population was 73%. Cryptococcosis was generally a late post-transplantation complication, with a median time to diagnosis (TTD) of 20 months after SOT with a significant proportion of patients presenting after 3 years (21).

In 2009, more than 65,000 patients were awaiting SOT (22). These patients have end-stage organ disease that is often complicated by multiple co-morbidities, and many of them are at risk for developing cryptococcosis while on the transplant waiting list. Patients with end-stage liver disease and cirrhosis are a subgroup at particularly high risk of cryptococcosis and may present with atypical manifestations, such as chronic peritonitis (4). As such, many patients develop cryptococcosis while on the waiting list for transplantation. Limited data are available on these patients, but most authorities agree that they can undergo transplantation once the infection is under good control

(23); that is, resolution of the signs and symptoms and negative culture results for *Cryptococcus* (24).

Current screening practices have a remarkable track record for maintaining safety in transplantation (25–27). Donor-derived cryptococcal infections occur, but this is distinctly uncommon. However, the occurrence of cryptococcosis in the first 30 days post-transplantation should raise the concern for either unrecognized pre-transplantation cryptococcosis or donor-derived cryptococcal disease (28, 29). If donor-derived infection is a concern, a key intervention is to ascertain the clinical status of other recipients of organs from the same donor and to test pre-transplantation serum from both donor and recipient for the presence of cryptococcal antigen (CrAg). Clusters of infection require notification of the organ procurement organization and of public health authorities to assist in the investigation (25). One of the more dramatic cases of donor-derived cryptococcosis is a recent report of cryptococcosis in which one liver and two renal transplant recipients developed cryptococcosis within 1 month of receiving organs from a donor with steroid-dependent sarcoidosis who died with an undifferentiated neurological syndrome (29). Two of the transplant recipients developed pneumonia, one developed meningitis, and all three developed cryptococcemia. None of these patients died from cryptococcosis. The recipients' fungal isolates where analyzed with multilocus sequence typing and found to be identical, thus confirming a common source of infection. Autopsy of the donor also confirmed the diagnosis of cryptococcosis. This case underscores the challenges faced by the transplantation team when confronted with a potential donor with an undifferentiated neurologic illness (26). Screening for cryptococcosis in the donor population has not been prospectively studied and is not recommended routinely, but it is prudent to remain alert to this possibility among potential organ donors with unexplained pulmonary and/or CNS disorders. SOT recipients are at high risk for nephrotoxicity associated with the concurrent use of amphoterian B (AmB) and calcineurin inhibitors (CINs). For this reason and the lack of any recent prospective data in the clinical trial setting, the Infectious Diseases Society of America (IDSA) (30) and American Society of Transplantation (5) recommend treating SOT recipients with disseminated, CNS, and severe pulmonary cryptococcosis with 2 weeks of induction therapy with the combination of a lipid formulation AmB plus flucytosine. For those patients with meningoencephalitis and a positive cerebrospinal fluid (CSF) culture at baseline, a repeat lumbar puncture is recommended before stopping this regimen; if CSF cultures remain positive, then a longer course of therapy is advised. For those SOT recipients with

isolated pulmonary disease, a lumbar puncture is recommended to exclude occult CNS involvement (30). Fluconazole 400 to 800 mg daily is recommended for induction therapy with mild to moderate pulmonary cryptococcosis.

The suggested consolidation and maintenance therapy in SOT recipients with CNS cryptococcosis is fluconazole (400 to 800 mg) for 8 weeks followed by fluconazole (400 to 200 mg) for 6 to 12 months (30). The use of this approach is associated with a very low risk of relapse, and is supported by data among a group of 79 patients with cryptococcosis in whom maintenance therapy was administered for a median of 183 days with relapse occurring in only 1 patient (1.3%) (31).

Reduction of immunosuppression in transplantation patients with cryptococcosis or other opportunistic infections seems to be a logical approach, but this should be done with caution. The goal of immunosuppressive reduction is to promote the eradication of the infection, but at the same time, it is important to minimize the risk of immune reconstitution inflammatory syndrome (24). Abrupt reduction of immunosuppressants can lead to immune reconstitution inflammatory syndrome (IRIS) with an associated risk of graft loss and worsening clinical symptoms. The recommended approach is to gradually reduce the corticosteroids before reducing the CINs and other immunosuppressive agents.

Many important questions remain pertaining to cryptococcosis in the transplantation patient: What is the optimal dose and duration of induction AmB for severe or life-threatening cryptococcosis, including CNS and pulmonary disease? What is the optimal strategy for immunosuppressive reduction after an episode of cryptococcosis to reduce the risk of IRIS? Are there easily available pre-transplantation screening approaches for cryptococcosis which are both feasible and effective? What is the optimal timing of transplantation in patients with a pre-transplantation diagnosis of cryptococcosis? Multicenter studies will be required to address the many key questions in this vulnerable population.

CRYPTOCOCCOSIS IN NON-HIV, NON-TRANSPLANT PATIENTS

Cryptococcosis is classically considered a systemic opportunistic mycosis, in that disease mostly occurs among patients who have a predisposing factor or underlying disease such as end-stage liver disease, renal insufficiency, sarcoidosis, and other conditions (2, 32–34). However, it can also affect patients who are phenotypically normal. Hence,

this is a heterogeneous population that ranges from apparently normal hosts to those with significant immunologic impairment including those on chemotherapy and/or immunosuppressive therapy, those with organ dysfunction and those with innate or acquired immunodeficiencies. It is difficult to draw conclusions about epidemiology, clinical presentation, prognosis, and outcomes given these varied host groups. Moreover, it is also challenging to tailor a treatment regimen that fits all patients (30).

Patients of particular interest in this group are those who have no known predisposing factors and develop severe pulmonary or extrapulmonary cryptococcosis. The subpopulation of these otherwise "normal" patients constitutes 17% to 22% of overall population in reported series of the NHNT patients. Although these patients seem to be a homogeneous group, they probably represent the congruence of subclinical innate or acquired immunodeficiencies. Outcomes and complications may be more severe in this group of otherwise normal patients, including more likely permanent neurologic sequelae such as stroke, blindness, deafness, and other focal cranial nerve abnormalities (35). A deeper understanding of the pathogenesis of cryptococcosis in this group could provide critical insights into mechanisms of disease and more effective therapy.

As currently understood, non-immunocompromised patients are generally more likely to develop pulmonary cryptococcosis as a sole manifestation of disease compared to immunocompromised hosts (32). Those who are least immunosuppressed tend to present with more localized findings on imaging, and granulomata with monocytic infiltration on histopathology. Those who are more immunosuppressed have a propensity to develop multifocal and/or diffuse findings on imaging, and histological findings which lack the presence of mononuclear inflammation and well-formed granulomata (36). Historically, NHNT patients presenting with meningoencephalitis tend to have higher CSF cell counts protein levels and lower glucose levels when compared to HIV-positive patients and transplant recipients (37). They are also less likely to be India ink–positive than their HIV counterparts.

In a recent retrospective study at our institution, we identified 302 patients diagnosed with cryptococcosis between 1996 and 2010 (38). Among these patients, 36% were HIV-positive, 28% were organ transplant recipients (OTRs), and 36% were NHNT patients. There were 39 phenotypically normal patients in the NHNT cohort. The demographics and underlying conditions of these patients are shown in Table 1. The mean TTD among NHNT patients was 68 days, significantly longer when compared to HIV-positive (22 days) and OTRs (26 days)

TABLE 1
Characteristics of 302 Patients With Cryptococcosis at UAB, 1996–2010

Characteristics	HIV N = 108 (%)	OTR N = 84 (%)	NHNT N = 110 (%)	*P* Value	Total Cohort N = 302 (%)
Mean age (± SD), years	39 (9.8)	54 (11.9)	56 (15.3)	<0.001	49 (12.6)
Male gender (%)	84 (78)	52 (62)	67 (61)	0.012	203 (67)
Caucasian (%)	23 (21)	66 (79)	83 (75)	<0.001	172 (57)
African American (%)	83 (77)	18 (22)	22 (20)	<0.001	123 (41)
Mean TTD, days	22	26	68	<0.001	40
Transplant type (%)					
Kidney	0 (0)	46 (55)	n/a	n/a	46 (15)
Liver	0 (0)	17 (20)	n/a	n/a	17 (6)
Heart	0 (0)	15 (18)	n/a	n/a	15 (5)
Lung	0 (0)	9 (11)	n/a	n/a	9 (3)
Pancreas	0 (0)	5 (6)	n/a	n/a	5 (2)
HSCT	0 (0)	2 (2)	n/a	n/a	2 (0.7)
Underlying disease (%)					
None	n/a	n/a	39 (36)	n/a	39 (13)
Steroids	0 (0)	73 (88)	27 (25)	<0.001	100 (33)
Renal Insufficiency/ESRD	3 (3)	36 (43)	7 (6)	<0.001	46 (15)
Cancer	1 (1)	5 (6)	31 (28)	<0.001	37 (12)
Diabetes mellitus	4 (4)	21 (25)	13 (12)	<0.001	38 (13)
Rheumatologic disease	0 (0)	1 (1)	7 (6)	0.008	8 (3)
Cirrhosis	0 (0)	11 (13)	5 (5)	<0.001	16 (5)
Site of infection (%)					
CNS	95 (88)	50 (60)	55 (50)	<0.001	200 (66)
Bloodstream	47 (44)	23 (28)	26 (24)	0.005	96 (32)
Pulmonary	13 (12)	31 (37)	44 (40)	<0.001	88 (29)
Cutaneous	4 (4)	8 (10)	2 (2)	0.033	14 (5)
Bone and joint	0 (0)	1 (1)	2 (2)	0.388	3 (1)
Soft tissue	0 (0)	2 (2)	1 (1)	0.251	3 (1)
Clinical presentation (%)					
Fever	44 (41)	33 (40)	31 (28)	0.119	108 (36)
Malaise	23 (21)	25 (30)	26 (24)	0.363	74 (25)
Weight loss	26 (24)	11 (13)	19 (17)	0.150	56 (19)
Headache	71 (66)	36 (43)	44 (40)	<0.001	151 (50)
Altered mental status	43 (40)	25 (30)	27 (25)	0.039	95 (31)
Visual changes	24 (22)	5 (6)	13 (12)	0.004	42 (14)
Cranial nerve palsy	9 (8)	3 (4)	9 (8)	0.364	21 (7)
Cough	15 (14)	20 (24)	25 (23)	0.137	60 (20)
Dyspnea	9 (8)	16 (19)	30 (28)	0.001	55 (18)
Diagnostics (%)					
Serum CrAg ≥ 1:512	40 (37)	24 (29)	16 (15)	0.001	80 (27)
CSF CrAg ≥ 1:512	40 (37)	17 (20)	20 (18)	0.003	77 (26)
CSF OP 25 cm H_2O	38 (35)	11 (13)	13 (12)	<0.001	62 (21)

TABLE 1—Continued

Characteristics	HIV N = 108 (%)	OTR N = 84 (%)	NHNT N = 110 (%)	*P* Value	Total Cohort N = 302 (%)
Mortality (%)					
90-day mortality	20 (19)	14 (17)	29 (27)	0.190	63 (21)
1-year mortality	28 (26)	20 (24)	38 (35)	0.193	86 (28)

Abbreviations: HIV, human immunodeficiency virus; OTR, organ transplant recipient; NHNT, non-HIV, non-transplant; TTD, time to diagnosis; HSCT, hematopoietic stem cell transplant; ESRD, end-stage renal disease; CNS, central nervous system; CrAg, cryptococcal antigen; CSF, cerebrospinal fluid; OP, opening pressure.

($P<0.001$). Compared to HIV-positive patients, OTRs and NHNT patients were less likely to have CNS involvement and cryptococcemia. Figure 1 shows the distribution of sites of involvement according to host immune status.

Among all 302 patients, 90-day mortality was 21%. Mortality was highest in the NHNT group (27%), but this did not reach statistical significance across groups ($P=0.190$). On univariate analyses, prognostic factors positively associated with 90-day mortality included cancer ($P=0.018$), fever ($P=0.031$), altered mental status ($P=0.001$), positive blood cultures ($P<0.001$), and high (≥1:512) serum CrAg ($P=0{\cdot}021$). Demographic and clinical features negatively associated

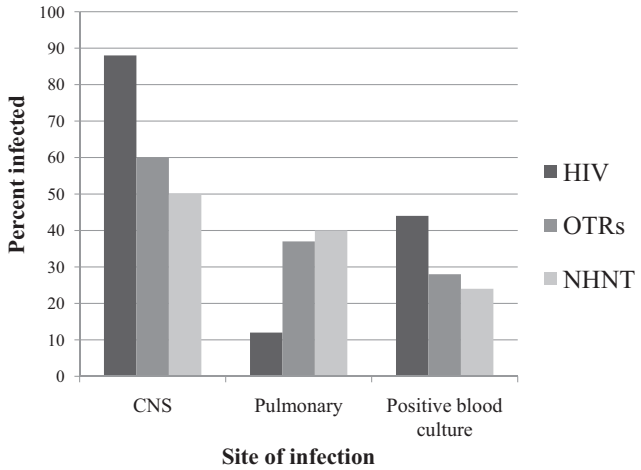

FIG. 1. Differential site of infection by host immune status among 302 patients with cryptococcosis at UAB, 1996–2010. (Abbreviations: HIV, human immunodeficiency virus; OTRs, organ transplant recipients; NHNT, non-HIV, non-transplant; CNS, central nervous system.)

with 90-day mortality were age <50 (P=0.020), headache at presentation (P=0.003) or cough (P=0.047), and pulmonary site of infection (P=0.027). In multivariable logistic regression analyses, cryptococcemia [odds ratio (OR) 5.09, 95% confidence interval (CI) 2.54–10·22; P<0.001], baseline opening pressure 25 cm H_2O (OR 2.93, 95% CI 1.25–6.88; P=0.013), and altered mental status (OR 1.96, 95% CI 0.98–3.91; P=0.057) were associated with increased odds of mortality. In contrast, age < 50 years (OR 0.42, 95% CI 0.20–0.92; P=0.029) and headache at presentation (OR 0.33, 95% CI 0.16–0.68; P=0·003) were protective (Table 2). Choice of induction therapy, either monotherapy or combination therapy, was not a significant prognostic factor.

A subanalysis of this cohort that compared features of 39 phenotypically normal patients to the other 263 immunocompromised patients yielded very interesting results. Table 3 lists the underlying conditions among the immunocompromised cohort, and shows that after HIV and organ transplantation, neoplasia, chronic glucocorticosteroids, and diabetes mellitus were most common. Table 4 describes the clinical presentation and sites of involvement for both cohorts, and shows that there are few differences between these groups, including clinical findings and the frequency of CNS cryptococcosis involvement. However, the time from initial symptoms to diagnosis was significantly different for the two groups: 34 days versus 81 days for immunocompromised and normal patients, respectively (P<0.001), showing a substantial delay in diagnosis. Other important differences between the two groups include higher mean CSF leukocyte counts (304 versus 79 cells/mm3) among "normal" hosts, higher serum CrAg titers for immunocompromised hosts, and higher 90-day and 1-year mortality for immunocompromised versus "normal" hosts. Finally, the need for a

TABLE 2
Results of Multivariable Analyses of Predictors of 90-day Mortality Among 302 Patients With Cryptococcosis at UAB, 1996–2010

Variable	OR	95% CI	P Value
Cryptococcemia	5.09	2.54–10.22	<0.001
Baseline CSF opening pressure 25 cm H_2O	2.93	1.25–6.88	0.013
Pulmonary cryptococcosis	0.44	0.18–1.07	0.070
Age < 50	0.42	0.20–0.92	0.029
Headache	0.33	0.16–0.68	0.003
Altered mental status	1.96	0.98–3.91	0.057
HIV versus NHNT	0.46	0.19–1.16	0.111
OTRs versus NHNT	0.46	0.21–1.05	0.111

Abbreviations: OR, odds ratio; CI, confidence interval; CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; NHNT, non-HIV, non-transplant; OTRs, organ transplant recipients.

TABLE 3
Features of IC Compared With Non-IC Subjects in 302 Patients With Crypto at UAB, 1996–2010

Characteristic	IC N = 263 (%)	Non-IC N = 39 (%)	*P* Value
Mean age, years (SD)	50 (14.8)	49 (14.9	0.815
Male gender	177 (67)	26 (67)	0.962
Caucasian	141 (54)	31 (79)	0.002
Underlying IC disease			
HIV	108 (41)	n/a	n/a
OTRs	84 (32)	n/a	n/a
Cancer	31 (12)	n/a	n/a
Chronic steroids	27 (10)	n/a	n/a
Diabetes mellitus	13 (5)	n/a	n/a
CKD / ESRD	7 (3)	n/a	n/a
Cirrhosis	5 (2)	n/a	n/a

Abbreviations: IC, immunocompromised; HIV, human immunodeficiency virus; OTRs, organ transplant recipients; CKD, chronic kidney disease; ESRD, end-stage renal disease; n/a, not available.

TABLE 4
Features of IC Compared With Non-IC Subjects in 302 Patients With Crypto at UAB, 1996–2010

Characteristic	IC N = 263 (%)	Non-IC N = 39 (%)	*P* Value
Site of involvement			
CNS	175 (67)	25 (64)	0.752
Bloodstream	95 (36)	1 (3)	<0.001
Pulmonary	72 (27)	16 (41)	0.067
Clinical presentation			
Headache	132 (50)	19 (49)	0.864
Fever	99 (38)	9 (23)	0.072
Altered mental status	88 (33)	7 (18)	0.053
Visual changes	34 (13)	8 (21)	0.209
Cough	51 (19)	9 (23)	0.607
Dyspnea	45 (17)	10 (26)	0.206
Mean time to diagnosis, days	34	81	<0.001

Abbreviations: IC, immunocompromised: CNS, central nervous system.

permanent CSF shunting procedure was significantly higher (44% versus 14%) in "normal" versus immunocompromised hosts (Table 5).

The treatment recommendations for the NHNT population are mostly based on data from clinical trials among HIV-infected patients or prospective studies performed on HIV-negative patients more than 20 years ago (30). There are little recent data regarding the duration of induction for therapy for NHNT patients who have meningoencephalitis. Most

TABLE 5
Features of IC Compared to Non-IC Subjects Among 302 Patients With Crypto at UAB, 1996–2010

Characteristic	IC N = 263 (%)	Non-IC N = 39 (%)	*P* Value
Serum CrAg ≥ 1:512	75 (29)	5 (13)	0.036
SF CrAg ≥ 1:512	70 (27)	7 (18)	0.237
Mean OP, cm H_2O	31	36	0.238
Mean CSF WBC, cells/mm^3	79	304	<0.001
Mean CSF glucose, mg/dL	48	37	0.053
Mean CSF protein, mg/dL	133	182	0.332
Permanent CSF shunt	24 (14)	11 (44)	<0.001
90-day mortality	59 (22)	4 (10)	0.081
1-year mortality	80 (30)	6 (15)	0.052

Abbreviations: IC, immunocompromised; CrAg, cryptococcal antigen; OP, opening pressure; CSF, cerebrospinal fluid; WBC, white blood cell.

experts favor 2 to 4 weeks of induction therapy with AmB with or without flucytosine, followed by step-down therapy to fluconazole 400–800 mg daily for up to 12 months, depending on the clinical response. Treatment for pulmonary cryptococcosis is directed towards improving the signs and symptoms of disease and to prevent dissemination of organisms to the CNS. All immunosuppressed patients with pulmonary cryptococcosis, regardless of symptoms, should be offered a lumbar puncture to exclude asymptomatic CNS cryptococcosis (30). Patients with severe manifestations of pulmonary disease should be treated similar to patients with meningoencephalitis, whereas most of those with mild to moderate disease can be affectively managed with fluconazole 400–800 mg daily as monotherapy for 6 to 12 months.

CRYPTOCOCCUS GATTII

Three studies from Australia and New Zealand (39–41) before the current BC/PNW outbreak suggest that *C. gattii* infection occurs dominantly in immunocompetent patients. In these series, the most common clinical presentation was meningoencephalitis followed by pulmonary involvement. The investigators also showed that, compared to patients with *C. neoformans* infections, patients with *C. gattii* infections were more likely to have a CNS or pulmonary cryptococcoma and to undergo surgical procedures to treat this complication. The most common molecular subtype reported in these series was VGI, and the mortality ranged between 0% and 15% (16).

The recent outbreak of *C. gattii* infections in BC and the US PNW has provided a somewhat different perspective into this infection.

Among the 218 cases recently described during the BC outbreak (6), 76.6% sought treatment for a respiratory syndrome, 7.8% for a CNS syndrome, and 10.1% for both respiratory and CNS syndromes. Approximately 40% were considered immunocompromised. Risk factors for *C. gattii* infection included age 50 years, smoking, corticosteroid use in the 3 months before onset, HIV infection, a history of cancer, and chronic lung disease (42). The molecular type VGIIa was responsible for 86.3% of cases, whereas VGIIb and VGI represented 7.3% and 6.5% of the cases, respectively. The observed mortality in this study was 8.7%.

C. gattii isolates in the US PNW differ somewhat from BC isolates, and the distribution of these molecular subtypes is as follows: VGIIa (50%), VGIIc (32%), VGIIb (10%), VGI (5%), and VGIII (3% (7). To date, VGIIc has not been reported outside the United States (16). A study led by the Centers for Diseases Control and Prevention (CDC) (8) divided the 96 cases of *C. gattii* reported in the United States during 2004 and 2011 into two groups: those who likely acquired infection in the PNW/BC and those who likely acquired it elsewhere. Given that the PNW outbreak is largely compromised of the molecular subtypes VGIIa, VGIIb, and VGIIc, these were considered outbreak strain infections. Non-outbreak strains (mostly VGI) were responsible for 100% of cases likely not acquired in PNW/BC cases whereas outbreak-strain infections were responsible for 94% of cases acquired in PNW/BC cases. Patients with outbreak-strain infection mainly presented with respiratory disease, and the majority of them had a pre-existing condition, most commonly chronic lung disease. On the other hand, almost all of the patients with a non-outbreak strain presented with CNS symptoms and only a third of them had a pre-existing condition. The mortality in this study was 33%, higher than in the previously published observations. An important observation from this study is that *C. gattii* affects patients without exposure to the BC/PNW, but it is a rare condition in the United States, causing both CNS and isolated pulmonary disease, in both immunocompetent and immunosuppressed patients. Active surveillance for cases of *C. gattii* is being undertaken to better define the prevalence of *C gattii* in the United States, and to address other important questions related to pathogenesis, relative virulence compared to *C. neoformans*, and optimal treatment (7, 8, 43, 44).

The diagnosis of *C. gattii* disease can be elusive; there is overlap in the epidemiology and clinical manifestations between *C. gattii* and *C. neoformans*. Both pathogens can affect immunocompetent and immunosuppressed hosts, and both cause CNS and pulmonary manifesta-

tions (6.8). Routine culture techniques and the CrAg assay do not distinguish between these infections. The use of CGB agar to distinguish *C. gattii* from *C. neoformans* is not routinely recommended in traditionally low incidence areas of the world, but should be considered in patients who present with intracranial cryptococcomas, for treatment- resistant cases, and when there has been exposure to areas endemic for the organism (16).

Recommendations from the IDSA (30) for the treatment of *C. gattii* infections are based on few prospective data. Patients with meningoencephalitis alone are treated with the same regimen as those patients with *C. neoformans*. Patients with multiple CNS cryptococcomas require longer duration of therapy, but the length of therapy is determined by clinical, mycologic, and radiographic response. Those patients with large intracranial cryptococcomas that are excised surgically may require shorter courses of antifungal therapy, but there is no consensus on this approach. For patients with pulmonary manifestations, treatment recommendations depend somewhat on the presence or absence of a cryptococcoma(s) and its size. Those with smaller cryptococcomas can be treated following the same recommendations as for *C. neoformans*, whereas those with large cryptococcomas may be treated with a combination of AmB and flucytosine for 4 to 6 weeks followed by 6 to 18 months of fluconazole. Thoracotomy for excision should be considered for patients in whom the lesions are unimproved after 4 to 6 weeks of therapy. These recommendations are not based on prospective data, but rather case reports and small case series. Intimately related to recommendations for the treatment is the report of higher minimum inhibitory concentrations (MICs) to selected azoles for *C. gattii*, although there is some inconsistency between the different studies, possibly due to variations between species and molecular types/subtypes and geographic regions. These recent studies show that the expanded-spectrum triazoles, including voriconazole, posaconazole, and isavuconazole (not FDA approved), have low MICs versus *C. gattii*. Unfortunately, there are few data correlating these antifungal MICs and clinical outcomes.

SUMMARY

A careful examination of cryptococcosis in non–HIV-infected patients provides important insights into the epidemiology, pathogenesis, treatment, and outcome of infections in this heterogeneous group of patients who have not been studied in detail in the last 2 decades. A critical analysis of cryptococcosis in these patients compared to those HIV-infected patients suggest substantial differences in terms of natural

history, clinical course, diagnosis, and outcome among these patients. As we explore cryptococcosis more deeply in non–HIV-infected patients, especially among those who are otherwise normal, one must ask the following questions: Apart from strain variation and different mating types, what determines clinical expression of disease in otherwise normal hosts? Why do these patients fare no better, and perhaps worse, than their immunocompromised counterparts? Is it reasonable to assume that treatment paradigms for HIV-infected patients apply to non-immunocompromised patients? Is there a role for immunomodulation (e.g., glucocorticosteroids or interferon gamma) as part of initial adjunctive therapy?

Among transplant recipients with cryptococcosis, we are particularly challenged due to the lack of prospective trials examining alternative approaches to therapy and prevention in SOT recipients. The role of CINs in the pathogenesis and outcomes of cryptococcosis are must be more clearly defined. There is a significant need for prospective trials to assess therapy outcome, risk factor assessment, and the influence of immunosuppressive therapy among transplant recipients. Finally, we must explore ways to identify patients pre-transplantation who are at particularly high risk for post-transplantation cryptococcosis.

The *C. gattii* outbreak in the PNW has also raised critically important epidemiologic and clinical questions: How is *C. gattii* spreading throughout the PNW? What are the geographic and ecological limitations to further spread? How prevalent is *C. gattii* in other regions of the United States? Who is at risk, and why do otherwise normal individuals seem to be a prime target for clinically apparent infections?

These questions are not only critical to the basic understanding of pathogenesis if this infection, but are quite relevant clinically. An enhanced understanding of these and other fundamental questions has the potential to impact millions of lives affected by this emerging fungal pathogen.

ACKNOWLEGDMENTS

I am indebted to my professional colleagues and friends, Kyle D. Brizendine, MD, John W. Baddley, MD, and Ricardo LaHoz, MD, for their efforts in assimilating and analyzing these clinical data. It is a privilege to work with each of you.

REFERENCES

1. Park BJ, Wannemuehler KA, Marston BJ, et al. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS* 2009;23(4):525–30.
2. Pappas PG, Perfect JR, Cloud GA, et al. Cryptococcosis in human immunodeficiency

virus-negative patients in the era of effective azole therapy. *Clin Infect Dis* 2001;33(5):690–9.
3. Dromer F, Bernede-Bauduin C, Guillemot D, Lortholary O. Major role for amphotericin B-flucytosine combination in severe cryptococcosis. *PLoS ONE* 2008,3:1–9.
4. Singh N, Husain S, De Vera M, et al. Cryptococcus neoformans infection in patients with cirrhosis, including liver transplant candidates. *Medicine (Baltimore)* 2004; 83(3):188–92.
5. Singh N, Forrest G, AST Infectious Diseases Community of Practice. Cryptococcosis in solid organ transplant recipients. *Am J Transplant* 2009;9(suppl 4):S192–8.
6. Galanis E, MacDougall L. Epidemiology of *Cryptococcus gattii*, British Columbia, Canada, 1999–2007. *Emerg Infect Dis* 2010;16:251–7.
7. MacDougall L, Kidd SE, Galanis E, et al. Spread of *Cryptococcus gattii* in British Columbia, Canada, and detection in the Pacific Northwest, USA. *Emerg Infect Dis* 2007;13:42–50.
8. Centers for Disease Control and Prevention (CDC). Emergence of *Cryptococcus gattii*&em;Pacific Northwest, 2004–2010. *MMWR Morb Mortal Wkly Rep* 2010; 59:865–8.
9. Harris JR, Lockhart SR, Debess E, et al. *Cryptococcus gattii* in the United States: clinical aspects of infection with an emerging pathogen. *Clin Infect Dis* 2011;53(12):1188–95.
10. Baddley JW, Dismukes WE. Cryptococcosis. In: Kauffman CA, Pappas PG, Sobel JD, Dismukers WE, eds. *Essentials of Clinical Mycology. 2nd ed.* New York: Springer; 2011:207–26.
11. Ikeda R, Shinoda T, Fukuzawa Y, et al. Antigenic characterization of *Cryptococcus neoformans* serotypes and its application to serotyping of clinical isolates. *J Clin Microbiol* 1982;36:22–9.
12. Franzot SP, Salkin IF, Casadevall A. *Cryptococcus neoformans* var. grubii: Separate varietal status for *Cryptococcus neoformans* serotype A isolates. *J Clin Microbiol* 1999;37:838–40.
13. Sanfelice F. Contributo alle morfologia e biologia dei blastomiceti che si sviluppano nei succhi di alcuni frutti. *Ann Isto Igiene R Univ Roma* 1894;4:463–95.
14. Kwon-Chung KJ, Boekhout T, Fell JW, et al. Proposal to conserve the name *Cryptococcus gattii* against *C. hondurianus* and *C. bacillisporus (Basidiomycota, Hymenomycetes, Trenomellomycetidae*). *Taxon* 2002;51:804–6.
15. Sorrell TC, Chen SC, Ruma P, et al. Concordance of clinical and environmental isolates of *Cryptococcus neoformans* var. *gattii* by random amplification of polymorphic DNA analysis and PCR fingerprinting. *J Clin Microbiol* 1996;34:1253–60.
16. Byrnes EJ 3rd, Bildfell RJ, Frank SA, et al. Molecular evidence that the range of the Vancouver Island outbreak of *Cryptococcus gattii* infection has expanded into the Pacific Northwest in the United States. *J Infect Dis* 2009;199(7):1081–6.
17. Kwon-Chung KJ, Polacheck I, Bennett JE. Improved medium for separation of *Cryptococcus neoformans* var. *neoformans* (serotypes A and D) and *Cryptococcus neoformans* var. *gattii* (serotypesB and C). *J Clin Microbiol* 1982;15:535–7.
18. Kwon-Chung KJ, Bennett JE. High prevalence of *Cryptococcus neoformans* var. *gattii* in tropical and subtropical regions. *Zentralbl Bakteriol Mikrobiol Hyg [A]* 1984;257:213–8.
19. Kwon-Chung KJ, Bennett JE. Epidemiologic differences between the two varieties of *Cryptococcus neoformans*. *Am J Epidemiol* 1984;120(1):123–30.
20. Stephen C, Lester S, Black W, et al. Multispecies outbreak of cryptococcosis on southern Vancouver Island, British Columbia. *Can Vet J* 2002;43(10):792–4.
21. Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ

transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Clin Infect Dis* 2010;50(8):1101–11.
22. Organ Procurement and Transplantation Network and Scientific Registry of Transplant Recipients 2010 data report. *Am J Transplant* 2012;12(suppl 1):1–156.
23. Sifri CD, Sun HY, Cacciarelli TV, et al. Pretransplant cryptococcosis and outcome after liver transplantation. *Liver Transpl* 2010;16(4):499–502.
24. Singh N. How I treat cryptococcosis in organ transplant recipients. *Transplantation* 2012;93(1):17–21.
25. Grossi PA, Fishman JA; AST Infectious Disease Community of Practice. Donor-derived infections in solid organ transplant recipients. *Am J Transplant* 2009;9(suppl 4):S19–26.
26. Grossi PA, Costa AN, Fehily D, et al. Infections and organ transplantation: new challenges for prevention and treatment&em;a colloquium. *Transplantation* 2012;93(suppl 5):S4–39.
27. Fishman JA, Greenwald MA, Grossi PA. Transmission of infection with human allografts: essential considerations in donor screening. *Clin Infect Dis* 2012;55(5):720–7.
28. Sun HY, Alexander BD, Lortholary O, et al. Unrecognized pretransplant and donor-derived cryptococcal disease in organ transplant recipients. *Clin Infect Dis* 2010;51(9):1062–9.
29. Baddley JW, Schain DC, Gupte AA, et al. Transmission of *Cryptococcus neoformans* by organ transplantation. *Clin Infect Dis* 2011;52(4):e94–8.
30. Perfect JR, Dismukes WE, Dromer F, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2010;50(3):291–322.
31. Singh N, Lortholary O, Alexander BD, et al. Antifungal management practices and evolution of infection in organ transplant recipients with *cryptococcus neoformans* infection. *Transplantation* 2005;80(8):1033–9.
32. Kiertiburanakul S, Wirojtananugoon S, Pracharktam R, et al. *Cryptococcosis* in human immunodeficiency virus-negative patients. *Int J Infect Dis* 2006;10(1):72–8.
33. Jongwutiwes U, Sungkanuparph S, Kiertiburanakul S. Comparison of clinical features and survival between cryptococcosis in human immunodeficiency virus (HIV)-positive and HIV-negative patients. *Jpn J Infect Dis* 2008;61(2):111–5.
34. Yuchong C, Fubin C, Jianghan C, et al. *Cryptococcosis* in China (1985–2010): review of cases from Chinese database. *Mycopathologia* 2012;173(5–6):329–35.
35. Ecezit IZ, Clancy CJ, Schmalfuss IM, et al. The poor prognosis of central nervous system *cryptococcosis* among nonimmunosuppressed patients: a call for better disease recognition and evaluation of adjuncts to antifungal therapy. *Clin Infect Dis* 2006;42:1443–7.
36. Brizendine KD, Baddley JW, Pappas PG. Pulmonary *cryptococcosis*. *Semin Respir Crit Care Med* 2011;32(6):727–34.
37. Shaunak S, Schell WA, Perfect JR. Cryptococcal meningitis with normal cerebrospinal fluid. *J Infect Dis* 1989;160(5):912.
38. Brizendine KD, Baddley JW, Pappas PG. Predictors of mortality and differences in clinical features among patients with cryptococcosis according to immune status. *PLoS ONE* 2013;8(3):e60431.
39. Chen S, Sorrell T, Nimmo G, Speed B, Currie B, Ellis D, et al. Epidemiology and host- and variety-dependent characteristics of infection due to *Cryptococcus neoformans* in Australia and New Zealand. Australasian Cryptococcal Study Group. *Clin Infect Dis* 2000;31:499–508.
40. Mitchell DH, Sorrell TC, Allworth AM, Heath CH, McGregor AR, Papanaoum K, et

al. Cryptococcal disease of the CNS in immunocompetent hosts: influence of cryptococcal variety on clinical manifestations and outcome. *Clin Infect Dis* 1995;20:611–6.
41. Speed B, Dunt D. Clinical and host differences between infections with the two varieties of *Cryptococcus neoformans*. *Clin Infect Dis* 1995; 21:28–34.
42. MacDougall L, Fyfe M, Romney M, Starr M, Galanis E. Risk factors for *Cryptococcus gattii* infection, British Columbia, Canada. *Emerg Infect Dis* 2011;17:193–9.
43. Marr KA. *Cryptococcus gattii*: the tip of the iceberg. *Clin Infect Dis* 2011; 53(12):1196–8.
44. Harris JR, Lockhart SR, Chiller T. Letter re: Marr editorial. *Clin Infect Dis* 2012;54(7):1038–9.

DISCUSSION

Wenzel, Richmond: Pete thanks so much, very interesting. You've raised a lot of questions; one, in terms of the outcome being different. With a delay in diagnosis, it seems like you might be just seeing late disease. Are there clinical correlates? For example, more visual disturbances, more fundoscopic examination differences, more brain edema on CT? A second question, if I can, are these people really normal or different from somebody? For example, are they outside more often than other people? Are they training for "an Iron Man Triathlon" in which, you know, your immune functions actually deteriorate? And what are you doing to measure immunities, particularly cell-mediated immunity, to see that maybe they look normal but, in fact, there is some other issue going on?

Pappas, Birmingham: Those are good questions. First, I think duration of symptoms and delayed diagnosis are keys. Also, the host immune response is really important: higher rates of blindness, deafness, and shunting are probably markers for more exuberant inflammation. If you've heard their stories, these patients show up with headache oftentimes for weeks or months before a clinician thinks about doing an LP, and often it's when they lose vision or hearing that somebody manages to perform an LP. It is as if they find cryptococcal disease almost by accident. So, I think length of symptoms has something to do with the rate of complications. Their inflammatory response, as you might expect, is more vigorous.

As to the genetics question, we are working with two groups; Lise-Anne Pirofski's group at Einstein, and we are beginning to provide her with DNA and cells from all of our non-HIV patients with cryptococcosis. We have the patient population, she has the technology, and we are beginning to work together in a cooperative arrangement. We continue to work with CDC and are trying to generate a bank of specimens from these types of patients.

As to your question regarding specific patient activities that might be risky, the majority of the people we see are pretty inactive. This is Alabama, and we are the obesity capital of the country, and most of these people, honestly, are not particularly active. We did a study of risk factors for cryptococcosis in HIV-infected patients with CDC years ago, and the only two things that trended toward significance were black race, and being in an outdoor occupation. We couldn't correlate risk with any specific activity or behavior.

Mushlin, New York: Really interesting epidemiologic data and important particularly in this endemic setting that you are dealing with. You know, the long duration of symptoms before diagnosis and the lower mortality rate in the non-immunocompromised patients really suggests to me that there may be a reservoir of unrecognized disease in

ambulatory patient populations and in primary care community-based settings. In Alabama, are there any data on that or are you looking more extensively in those settings for unrecognized cryptococcal disease and a symptom complex that may be related to it?

Pappas, Birmingham: Basically, what I can share with you about Alabama and cryptococcosis is this: We gathered the data in early 1990s, when we polled all of the ID physicians in the state, making the assumption that anyone with cryptococcal disease would be eventually be seen or reported to an ID physician. There were about 10 ID physicians outside of Birmingham at that time, and we were able to calculate a statewide incidence of about 2/100,000 persons. These were mostly non–HIV-infected patients. We published these data in the *American Journal of Epidemiology* in 1994, but unfortunately, the state didn't take a great deal of interest in the data, so we have never really been able to make it into a reportable disease statewide or to alert physicians to the importance of this disease. To answer your question, I don't know how much goes undiagnosed, but I can tell you with confidence that late diagnosis relates to poor recognition on the part of treating physicians.

Mackowiak, Baltimore: Very interesting presentation, Peter. You alluded to this question and answered to it several times but have never addressed it directly. Why is cryptococcosis so common in Alabama?

Pappas, Birmingham: That's a good question.

Mackowiak, Baltimore: I mean, honestly, is it the soil, is it the moisture?

Pappas, Birmingham: The closer one gets to the tropics, generally the more cryptococcal disease one encounters. To my knowledge, there are no data for other southeastern states, but it would be interesting to see data from Mississippi and Georgia. Stan Chapman is here, and he could probably confirm that in Mississippi rates are similar to ours, but I don't know this. I don't think these have been measured, but I suspect they see about as much cryptococcal disease as we do. So, is it in the environment, is it in the soil? That certainly makes sense, but I just don't know. I can tell you that the *C. gattii* epidemic epidemiologists and the forestry service personnel in British Columbia have looked into this extensively and have found the epidemic strains in abundance in certain soil types and surrounding certain trees.

Hochberg, Baltimore: So, about two-thirds of your non-HIV non–organ transplant population were immunocompromised right? So, of that group, which I guess was around 70, how many of those were on glucocorticoids and/or TNF inhibitors.

Pappas, Birmingham: I have the information but I can't recall the numbers specifically. I can tell you that the bulk of them were steroid recipients. The next largest group was diabetics, and a smaller group with TNF-alpha inhibitors, and fewer still with organ failure syndromes.

Hochberg, Baltimore: I was going to ask if the group that was on glucocorticoids or TNF inhibitors had a different clinical outcome.

Pappas, Birmingham: Too few of these patients for me to address that question accurately.

Baum, New York: A lot of the questions have dealt with the beginning of the disease and the prolonged presentation time. How about the end? In New York with lots of these patients being HIV-positive and having clinical AIDS, we've gotten used to treating them for life. I am old enough to remember cryptococcosis before AIDS and, you know, when they got better and the second LP showed a marked decrease in the lymphocytes, we could no longer detect much antigen, we stopped. Are you treating these patients, other than the AIDS patients, for life?

Pappas, Birmingham: We don't treat most AIDS patients for life any longer. We treat the AIDS patients until their CD4s are 100 or 150 cells/mm^3 consistently, and then

withdraw therapy. If their cultures are negative, if the CD4 count is above this level, and they've have had several months of antiretroviral therapy, then we stop therapy. We don't have a similar immunologic marker in transplant patients, but our rule of thumb is to continue therapy for 6 to 12 months, reassess, and then stop therapy if the patient is stable and without evidence of relapsing or persistent disease. The only persons who traditionally go beyond 12 months of therapy are those who continue to have pulmonary radiographic findings that are worrisome for persistent disease.

Baum, New York: And are the ones who are dying dying during therapy?

Pappas, Birmingham: I haven't shown you mortality curves, but most death due to cryptococcus occurs within 90 days. The curves really flatten out after that, but then death is generally due to underlying disease.

Donowitz, Charlottesville: *C. gattii* is supposed to be harder to treat, so you treat for longer, and there are more relapses. Is that due to differences in susceptibility or interaction with host defense? Do you have a sense of that?

Pappas, Birmingham: It's not clear. We are in the midst of gathering data from six international sites pertaining to cryptococcosis. We are going to collate data from around 1200 cases, about 700 *C. neoformans* and 500 *C. gattii* cases with the purpose of comparing clinical and microbiologic data for these two organisms. As for antifungal susceptibility, it does appear that certain outbreak strains are more resistant to fluconazole. Posaconazole or voriconazole may be the best alternatives in these settings when fluconazole fails.

Oates, Nashville: *C. gattii* is a tropical organism that now is appearing in North America. Is this an example of the type of pathogen distribution change that might be expected with climate change?

Pappas, Birmingham: Not necessarily. No one knows exactly how this occurred, but this new strain certainly arose from an unusual mating event. Fungi are sexual organisms and there are different mating types; it is hypothesized that an imported strain from Australia or the Far East mated with a local (Vancouver Island) cryptococcus, a new hybrid resulted with increased virulence, and the rest is history. These VGII a, b, and c strains really are quite unique. Who knows how this occurred? Eucalyptus trees are all up and down the West Coast, and many were brought from Australia. Other wood products come to the West Coast from the Far East. In a way, it's surprising that it has not happened before now. What's most surprising to me is that these new strains of *C. gattii* have arisen on Vancouver Island rather than a tropical milieu. I do like to relate lots of things to global warming, but this not one of those events, in my opinion. At least that I can't really make a good connection between this event and global warming.

Richardson, Richmond: What functions do they have aside from infecting Alabamians and Pacific Northwesterners?

Pappas, Birmingham: I don't understand the question.

Richardson, Richmond: What makes them thrive beside humans?

Pappas, Birmingham: Cryptococcus is like aspergillus the other fungi. . .their main purpose is to degrade organic material. That is their basic function, and something that all fungi have in common. Cryptococcus was first cultured from peach juice in Italy in the 1890s, and it was considered to be a non-pathogen. At the time it was called torulopsis, and felt to be a good fermenting agent. It didn't work well as a fermenting agent; it's not an important plant pathogen, so it must serve some other purpose in nature. I hope this addresses your question. Thank you all for your kind attention and great questions.